

## *Reference 1*

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## Radiation resistance of *Salmonella*

Donald W. Thayer, Glenn Boyd, Wayne S. Muller, Carol A. Lipson, Walter C. Hayne and  
Steven H. Baer

U.S. Department of Agriculture, ARS, Eastern Regional Research Center, Philadelphia, PA, U.S.A

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### SUMMARY

The ionizing radiation resistances of six *Salmonella* species were examined. The experimental variables were the suspending medium, the presence or absence of air, and the temperature during the irradiation process. *S. typhimurium* ATCC 14028, *S. enteritidis* ATCC 9186, *S. newport* ATCC 6962, *S. dublin* ATCC 15480, *S. anatum* ATCC 9270, and *S. arizonae* ATCC 29933 were suspended in phosphate buffer (0.1 M, pH 7.0), brain heart infusion broth (BHI) or mechanically deboned chicken and exposed to gamma radiation from cesium-137 at 0.12 kGy per min. The radiation resistance of the *Salmonella* increased approximately two-fold when assayed in sterile mechanically deboned chicken rather than in buffer or BHI. The average radiation (0.30 to 1.20 kGy) D-value for all six *Salmonella* strains was 0.56 kGy in mechanically deboned chicken. *S. enteritidis* was significantly more resistant to ionizing radiation than the other five strains of *Salmonella* tested on mechanically deboned chicken. The temperature of irradiation but not the presence or absence of air significantly influenced the survival of *S. typhimurium* and *S. enteritidis* in mechanically deboned chicken. Treatment of chicken meat with ionizing radiation would be an effective means for control of *Salmonella* contamination.

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### INTRODUCTION

The Food Safety and Inspection Services (FSIS) of the U.S. Department of Agriculture has peti-

tioned the Food and Drug Administration for approval of irradiation pasteurization of retail packaged, frozen or fresh, uncooked poultry products. The petition lists the following as possible sources of ionizing radiation: cobalt-60, cesium-137, electron beam accelerators, and X-ray generators. The poultry products are to be irradiated in air-permeable packaging to an absorbed dose of 1.50-3.00

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Correspondence: U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118, U.S.A.

kGy to decrease the potential of food-borne illness from such food-borne pathogens as *Salmonella*, *Campylobacter* and *Yersinia*. This radiation dose range was chosen because higher doses might eliminate the normal microbial flora from the products and thereby increase the opportunity for *Clostridium botulinum* to grow and produce toxin. Air permeable packaging was required in an additional effort to prevent the growth of *C. botulinum*.

A number of previous investigators have examined the effects of ionizing radiation on *Salmonella* associated with poultry. Mulder et al. [9] examined chilled and deep-frozen broiler carcasses and found 2 to 1400 colony forming units of *Salmonella* per 100 g of skin. Recently, FSIS has indicated that as many as 30% of the poultry carcasses processed in the U.S.A. may be contaminated with *Salmonella*. The contamination levels are not thought to be greatly different from those found by Mulder et al. [9] in the Netherlands, who reported that irradiation to a total dose of 2.5 kGy was highly effective in destroying the *Salmonella*. However, a radiation dose of 2.5 kGy might not destroy all the *Salmonella* in poultry carcasses since 7.0 kGy was necessary to destroy *Salmonella panama* present on poultry carcasses [7].

Mechanically deboned poultry meat is frequently shipped long distances to be further processed into foods. This product would seem to be particularly well suited for irradiation pasteurization, yet very little data exist on the effects of ionizing radiation on its microbial flora. This product is usually shipped to the processor in 20 to 40 pound lots which would be expected to be anaerobic a centimeter or more from the surface of the meat. The purpose of this study was to establish the response of six common food-borne *Salmonella* to ionizing radiation in commercial mechanically deboned chicken meat under various conditions.

## MATERIALS AND METHODS

### *Organisms and growth conditions*

*Salmonella enteritidis* ATCC 9186, *S. newport* ATCC 6962, *S. dublin* ATCC 15480, *S. anatum*

ATCC 9270, *S. arizonae* ATCC 29933, and *S. typhimurium* ATCC 14028 were used in these studies. Each strain was maintained and cloned on Difco Laboratories Tryptic Soy Agar (TSA) with incubation at 35°C. Culture purities and identities were verified with Gram stains and API 20 E strips. One milliliter from an overnight (15–18 h) culture of the organism in Baltimore Biological Laboratories Trypticase Soy Broth (TSB), incubated at 35°C, was used to inoculate 100 ml of TSB or Difco Laboratories Brain Heart Infusion Broth (BHI) in 500-ml baffled shake flasks. The flasks were then shaken (150 rpm) on a gyratory shaker for 16 h at 35°C. This culture was used directly as an inoculum for those studies involving potassium phosphate buffer (pH 7.0, 0.15 M) or BHI broth. A 10-fold concentrated inoculum was prepared for studies involving mechanically deboned chicken meat by growth of the appropriate *Salmonella* species as described above in TSB and pelleting of the cells by centrifugation and resuspension in one-tenth volume of 0.1% peptone (Difco).

### *Phosphate buffer and brain heart infusion broth studies*

Inocula of 0.1 ml of the appropriate salmonellae strain were used for 5.0 ml of pH 7.0, 0.15 M potassium phosphate buffer or BHI broth contained in 10.0 ml screw cap culture tubes. Three replicate tubes were prepared for each treatment. To provide a comparison to results obtained with mechanically deboned meat, experiments were conducted with *S. enteritidis* 9186 and *S. typhimurium* 14028 in which 5.0 ml amounts of inoculated buffer or BHI broth were heat-sealed in Stomacher<sup>®</sup> 400 polyethylene bags either containing air or in vacuo. These bags were then vacuum packed in American Can Company Freshtuff bags (oxygen transmission 0.6–0.8 cc/100 sq in/24 h @ 37.8°F and 90% R.H.).

### *Mechanically deboned chicken meat studies*

Mechanically deboned chicken meat was obtained from a commercial manufacturer of poultry frankfurters. The meat was received in two commercial 18 kg lots and consisted of approximately 90% rib and 10% back meat. The proximate analy-

sis of this product was 63.1% moisture, 25.7% fat, and 11.4% protein. The chicken meat, subdivided into  $50.0 \pm 0.05$  g lots, was first vacuum sealed in Stomacher® 400 polyethylene bags, and then vacuum sealed in American Can Company Freshstuff bags. These replicate samples of the mechanically deboned chicken meat were then stored at  $-20^{\circ}\text{C}$  until used. The frozen chicken meat samples were further cooled to  $-40^{\circ}\text{C}$  and then irradiated at the same temperature with gamma radiation to an absorbed dose of 42 kGy. This treatment effectively eliminated any natural microbial flora from the product as confirmed by plate count. The radiation-sterilized chicken was stored at  $-20^{\circ}\text{C}$  until used.

The radiation-sterilized mechanically deboned chicken was inoculated with approximately  $10^9$  *Salmonella* cells per gram of chicken meat. The inoculated meat was then mixed thoroughly with a Stomacher® 400 for 90 S and aseptically divided into  $5.0 \pm 0.05$  g samples which were packaged in Stomacher® bags. The Stomacher® 400 bags (polyethylene) were heat sealed with air in the bag or in vacuo at  $-1.0$  bar. All bags were then vacuum packaged in American Can Company Freshstuff bags. Three replicate inoculated samples were irradiated at each dose. The gamma radiation doses used for the survival studies in chicken meat were 0, 0.30, 0.60, 0.90, 1.20, 1.50, 1.80, 2.70 and 3.60 kGy. All six strains were tested over the dose range of 0 to 1.50 kGy; *S. enteritidis* and *S. typhimurium* were tested to a maximum dose of 3.6 kGy.

#### *Irradiation*

Samples were irradiated in a self-contained cesium-137 gamma radiation source (140 708 Ci) producing a dose rate of 0.12 kGy per min. The dosimetry and dose distribution for this radiation source were described by Shieh et al. [12]. Routine dosimetry was conducted with ferrous sulfate/cupric sulfate dosimeters [4]. The samples were brought to the desired temperature before irradiation and this temperature was maintained  $\pm 2^{\circ}\text{C}$  during irradiation by the injection of the gas phase from liquid nitrogen. The samples were placed in a uniform portion of the radiation field and arranged to minimize any differences in the radiation dose. The mean devia-

tion of the absorbed dose from the target dose was 0.038 kGy with a standard error of 0.018 kGy.

#### *Microbiological assay*

The samples were assayed for colony forming units (CFU) by standard pour plate procedures with serial dilutions in 0.1% Difco Bacto peptone. The pour plates were prepared using TSA and incubated for 24 h at  $35^{\circ}\text{C}$ . All studies were performed with three replicate samples for each treatment and three plate counts at the appropriate dilution giving 30 to 300 colony forming units (CFU) per Petri plate for each replicate sample. Bacterial colonies on pour plates were counted using a New Brunswick Scientific Biotrans® II automated colony counter. Results are reported as the logarithm of the surviving fraction of CFU ( $\log N/N_0$ ).

#### *Mathematical analysis*

For each experiment, the average ( $N$ ) of the CFU values for the three plate counts obtained for each replicate sample was determined and divided by the average of the three zero dose CFU values ( $N_0$ ) to give a value for survivors ( $N/N_0$ ). The log-survivor values ( $\log_{10}$  of  $N/N_0$ ) were used in the determination of the slope of the regression of the data by least squares analysis using the REG procedure of the SAS system for linear regression [1]. The  $N_0$  values were not used in the computation of the regressions to eliminate possible shoulder effects. At least four radiation dose levels were used in the calculation of each regression. Each D-value in Tables 1, 3, and 5 was determined from the regression of all values on one or more experiments, the D-value being the negative reciprocal of the slope of the individual regression of log-survivor against radiation dose. The slopes of the regressions were compared by analysis of covariance using the General Linear Model procedure of the SAS system for linear models [1].

## RESULTS

#### *Phosphate buffer*

The survival of *S. dublin* in pH 7.0 phosphate

Table 1

Effect of suspending medium, phosphate buffer, pH 7.0 or brain heart infusion broth, on the gamma radiation D-values\* of six strains of *Salmonella*

Strain	Phosphate buffer			Brain heart infusion		
	N <sup>b</sup>	D(kGy)	± SE	N <sup>a</sup>	D(kGy)	± SE
<i>S. anatum</i>	9	0.116	0.004	36	0.288	0.032
<i>S. arizonae</i>	39	0.184	0.024	18	0.244	0.023
<i>S. dublin</i>	18	0.267	0.013	18	0.341	0.011
<i>S. enteritidis</i>	45	0.172	0.022	18	0.264	0.016
<i>S. newport</i>	15	0.152	0.012	17	0.212	0.017
<i>S. typhimurium</i>	132	0.199	0.013	18	0.220	0.016

\* D-values calculated from least squares regression analysis of all determinations in range of 0.10 to 1.20 kGy.

<sup>b</sup> N represents the number of individual values used to compute the regression.

buffer was greater than any other strain (Table 1); however, only four comparisons of regression slopes revealed significant ( $P > 0.05$ ) differences (Table 2). The regressions of log of the survivors against dose were significantly different when the regression for *S. enteritidis* was compared with that of *S. arizonae*. Significant differences between regressions were also found for *S. newport* and *S. enteritidis*, *S. typhimurium* and *S. enteritidis*, and *S. typhimurium* and *S. newport*. Regression values for *S. enteritidis* cells suspended in buffer in the pres-

ence of air compared with those obtained in the absence of air were significantly different ( $P > 0.002$ ). *S. enteritidis* was more resistant to the effects of ionizing radiation in the presence of air. A similar result ( $P > 0.02$ ) was obtained with *S. typhimurium* irradiated in phosphate buffer in the presence and absence of air, but only when the comparison was made with results from a single experiment. As was the case with *S. enteritidis*, the *S. typhimurium* cells irradiated in the presence of air were more resistant to the radiation.

Table 2

Heterogeneity of slopes of regressions from which the D-values for the survival of *Salmonella* in phosphate buffer were computed. (Probability of obtaining a value greater than F when each slope is compared to each other slope)

Strain	1	2	3	4	5	6
1	X	0.6607	0.9482	0.1146	0.4655	0.9538
2		X	0.4404	0.0024	0.8139	0.3537
3			X	0.0563	0.4671	0.8353
4				X	0.0001	0.0019
5					X	0.0037
6						X

Strains: #1 = *S. anatum*, #2 = *S. arizonae*, #3 = *S. dublin*, #4 = *S. enteritidis*, #5 = *S. newport*, #6 = *S. typhimurium*.

#### Brain heart infusion broth

As expected, the radiation resistance of the *Salmonella* species was greater in more complex suspending media than in phosphate buffer (Tables 1 and 3). In phosphate buffer and BHI broth, *S. dublin* had the greatest D-value. The D-value for *S. dublin* in BHI broth was significantly ( $P < 0.05$ ) different from that of *S. enteritidis*, *S. newport* and *S. typhimurium* (Table 3). The D-value of *S. enteritidis* was significantly different from that of *S. typhimurium* only.

#### Mechanically deboned chicken

Because it is unknown what effect the normal microbial flora of this product might have on the sur-

Table 3

Heterogeneity of slopes of regressions from which the D-values for the survival of *Salmonella* in brain heart infusion broth were computed. (Probability of obtaining a value greater than F when each slope is compared to each other slope)

Strain	1	2	3	4	5	6
1	X	0.4352	0.3032	0.7184	0.0913	0.1433
2		X	0.0067	0.5086	0.2557	0.3933
3			X	0.0012	0.0001	0.0001
4				X	0.0411	0.0759
5					X	0.7237
6						X

Strains: #1 = *S. anatum*, #2 = *S. arizonae*, #3 = *S. dublin*, #4 = *S. enteritidis*, #5 = *S. newport*, #6 = *S. typhimurium*.

vival of a *Salmonella* species following ionizing radiation treatment, it was decided that the competition factor should be eliminated in initial studies. Extensive data indicate that poultry products that are radiation sterilized (42 kGy) in vacuo at  $-30^{\circ}\text{C}$  or lower are not significantly altered chemically or toxicologically [14]. This method was therefore chosen to provide sterile poultry meat for the study. Subsequent studies will then compare results obtained with similar non-sterile samples from the same lot of mechanically deboned meat.

The measured D-value for *S. enteritidis* was considerably greater than that of the other four *Salmonella* strains (Table 4) when irradiated in mechanically deboned chicken. The tendency to increased radiation resistance of salmonellae when suspended in more complex media is also evident. Comparisons of the regressions from the average values from Table 4 indicated that *S. enteritidis* was significantly different from *S. arizonae*, *S. newport*, and *S. typhimurium* but not from *S. anatum*, or *S. dublin* (Table 5). The D-value of *S. arizonae* was significantly different only from *S. enteritidis*, and the D-value of *S. newport* was significantly different only from that of *S. enteritidis* and *S. typhimurium* (Table 5).

D-values for *S. typhimurium* and *S. enteritidis* in mechanically deboned chicken were computed over dose ranges of 0.3 to 1.2 kGy and 0.3 to 2.7 kGy. Significant ( $P > 0.012$  for *S. enteritidis* and  $P > 0.026$  for *S. typhimurium*) decreases in the computed D-values were found when the range was extended to include 2.7 kGy radiation doses. (The bacterial suspensions were also exposed to doses of 3.6 kGy, but many had few and some no survivors and thus were excluded from the computations.) The results for *S. typhimurium* are illustrated in Fig. 1.

Although several experiments (Table 4) indicated greater lethality of ionizing-radiation in the pres-

Table 4

Effects of air or vacuum on the survival of *Salmonella* irradiated in mechanically deboned chicken

Strain	Air			Vacuum			Air and vacuum		
	D-value N	D(kGy)	$\pm$ SE	D-value N	D(kGy)	$\pm$ SE	D-value N	D(kGy)	$\pm$ SE
<i>S. anatum</i> <sup>a</sup>	24	0.542	0.187	24	0.500	0.133	48	0.520	0.114
<i>S. arizonae</i> <sup>a</sup>	12	0.421	0.024	12	0.470	0.050	24	0.444	0.057
<i>S. dublin</i> <sup>a</sup>	12	0.467	0.028	12	0.618	0.051	24	0.532	0.107
<i>S. enteritidis</i> <sup>a</sup>	24	0.772	0.154	24	0.774	0.078	48	0.773	0.095
<i>S. enteritidis</i> <sup>b</sup>	33	0.534	0.036	33	0.592	0.021	66	0.561	0.095
<i>S. newport</i>	24	0.436	0.147	12	0.374	0.016	35	0.378	0.030
<i>S. typhimurium</i> <sup>a</sup>	36	0.533	0.031	36	0.497	0.058	72	0.514	0.034
<i>S. typhimurium</i> <sup>b</sup>	51	0.387	0.010	48	0.394	0.020	99	0.390	0.010

<sup>a</sup> Survival D-values computed from the regression values from 0.30 to 1.20 kGy.

<sup>b</sup> The second set of values for *S. enteritidis* and *S. typhimurium* represent regression values for 0.30 to 2.70 kGy.

Table 5

Heterogeneity of slopes of regressions from which the D-values for the survival of *Salmonella* irradiated to an absorbed dose of 0.30 to 1.20 kGy in mechanically deboned chicken meat (air and vacuum packaged) were computed. (Probability of obtaining a value greater than F when each slope is compared to each other slope)

Strain	1	2	3	4	5	6
1	X	0.6607	0.9482	0.3146	0.4655	0.9538
2		X	0.4404	0.0024	0.8139	0.3537
3			X	0.0963	0.4671	0.8353
4				X	0.0001	0.0019
5					X	0.0037
6						X

Strains: #1 = *S. anatum*, #2 = *S. arizonae*, #3 = *S. dublin*, #4 = *S. enteritidis*, #5 = *S. newport*, #6 = *S. typhimurium*.

ence of air, statistical analysis did not confirm that the observations were significant in most cases. Exceptions were the D-values for *S. dublin* in vacuum packed and aerobically packed mechanically de-

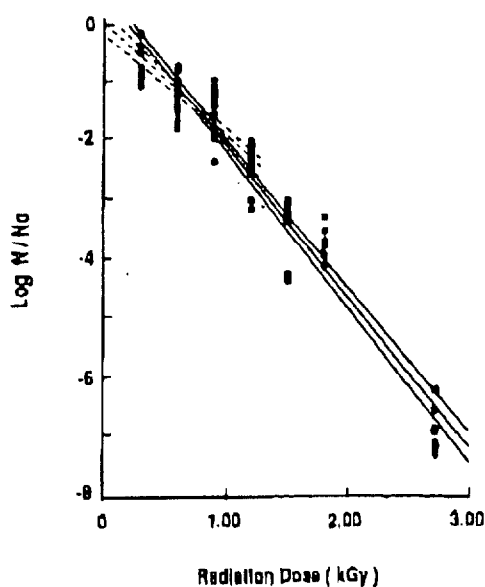


Fig. 1. Survivor ( $N/N_0$ ) plots for *S. typhimurium* with associated 95% confidence limits plotted between 0.30 and 1.20 kGy (---) and 0.30 and 2.70 kGy (—) in mechanically deboned chicken meat.

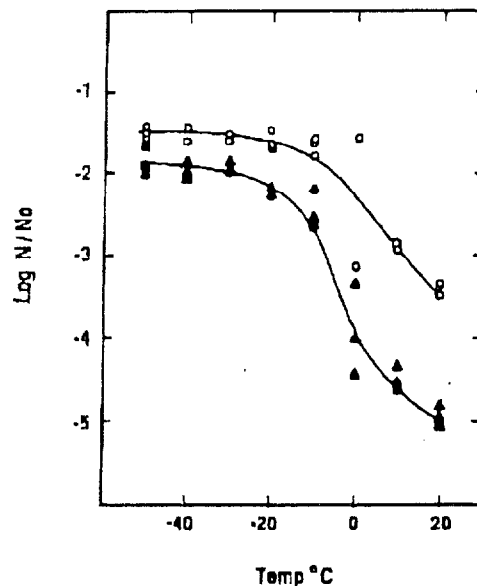


Fig. 2. The effect of irradiation temperature on the survival of *S. typhimurium* ( $\Delta$ ) and *S. enteritidis* (O) at an absorbed dose of 1.80 kGy in mechanically deboned chicken meat.

boned chicken which were significantly different ( $P > 0.01$ ). The regressions were significantly different at the  $P > 0.02$  level in one study of *S. typhimurium*.

The response of *S. enteritidis* and *S. typhimurium* to irradiation temperature at an absorbed dose of 1.80 kGy in mechanically deboned chicken is illustrated in Fig. 2. Both organisms were strongly protected against irradiation by temperatures below  $-20^\circ\text{C}$ . The much greater resistance of *S. enteritidis* to ionizing radiation compared to that of *S. typhimurium* is also evident.

## DISCUSSION

Because  $\text{H}_2\text{O}_2$  is formed during irradiation in the presence of oxygen [16] ionizing radiation might have increased lethality for bacteria in its presence. Several authors have reported that bacterial sensitivity to ionizing radiation could be reduced by eliminating oxygen from the suspending medium; the species studied included *Escherichia coli*, *Bacil-*

*lus anthracis*, and *Staphylococcus aureus* [3,5,10,13,15,17]. It has been demonstrated repeatedly [2,6] that molecular damage of DNA or nucleic acids is oxygen dependent. However, *Pseudomonas geniculata* and *Bacillus thermoacidurans* spores were equally sensitive to ionizing radiation whether or not oxygen was present [10]. In contrast with the D-value of 0.56 kGy for *S. typhimurium* reported by Previte et al. [11], we observed a D-value of 0.22 kGy for *S. typhimurium* irradiated in BHI broth.

The *S. typhimurium* D-value reported here for the range 0.3 to 1.2 kGy of 0.514 kGy is very similar to the values of 0.52–0.68 kGy reported by Previte et al. [11] for five different strains of this organism irradiated at 4°C in autoclaved chicken. The shift in the computed value that occurred when values were included for doses of 1.5 and 2.7 kGy was significant ( $P > 0.026$ ), and the data has an apparent shift (Fig. 1). However, the Previte et al. [11] computation was made from data that included 5.0 kGy. The authors conclude from the shift in computed D-values with increased radiation dose range that one should use care in extrapolating D-value determinations to cover doses greatly in excess of that experimentally evaluated. For example, the 12 D value for *S. enteritidis* using a D-value of 0.773 kGy would be 9.28 kGy; whereas, the 12 D value computed using a D-value of 0.561 would be 6.75 kGy. The current results indicate that the minimum radiation dose of 1.5 kGy proposed by FSIS would produce roughly a 2.8-decimal reduction and the maximum dose of 3.0 kGy a 5.3 decimal reduction of *S. enteritidis* in mechanically deboned chicken. Mulder [7] estimated that a dose of 7.0 kGy would be required to ensure the absence of *Salmonella panama* from deep-frozen broiler carcasses. Our observation that sharply increased survival occurred when the meat samples were irradiated in the frozen state requires further investigation, but the work of Mulder [8] indicated that following a radiation dose of 2.5 kGy that the few remaining viable *Salmonella* on poultry carcasses died when the carcass was stored at  $-18^{\circ}\text{C}$ . Since the numbers reported by Mulder et al. [9] did not exceed 14 *Salmonella* per gram of skin this dose should be entirely adequate for control of this pathogen.

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